FUSIFORM BACILLI. ISOLATION AND CULTIVATION.*†

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Although an extensive literature exists on the fusiform bacillus, there are recorded no simple methods for the isolation and cultivation of these bacilli. The following methods were worked out in our endeavor to isolate a large number of strains for a comparative study.

The isolation from shake and surface cultures was tried. These methods were found unsatisfactory. The possibility of success depends on an abundance of bacilli in the original material or the examination of smears from colonies till one might chance on a fusiform colony. The use of surface growths was not seriously considered, as we were endeavoring to dispense with the use of pyrogallic acid. Shake cultures in capillary tubes were then made so that the character of the colonies could be studied under the microscope. This was also found unsatisfactory. The cultivation in agar contained between two layers of glass was then tried. For this purpose the two halves of the petri dish were sterilized so that the bottom was placed in the inverted cover. The inoculated ascitic agar was poured into the cover and the bottom of the dish was laid on top of the agar while it was still fluid. A very few threaded colonies were noted and fishing from these gave fusiform bacilli. This gave us the basis for future work.

The original material is suspended in about 5 to 7 c.c. of ascitic fluid or horse serum, and successive dilutions made in a series of tubes containing the same amount. In making the dilutions a pipette is used carrying over about 0.1 to 0.2 c.c. to make the dilutions gradual. About 15 c.c. of fluid agar is then poured into the tubes and the mixture poured in the manner already described (Fig. 1). During incubation the plates should be covered with paper to limit air contamination of the rim of exposed agar or the

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† This study had its inception in the interest of our associate, Dr. Matthias Nicoll, Jr., in the clinical conditions in which this organism is found. We are indebted to him for our material.

rim may be paraffined, which would also lessen the drying-out of the medium.

After incubation for 48 to 72 hours the upper layer of the dish is wrenched loose and the typical colonies searched for. These are characterized by threadlike outgrowths from one or both sides

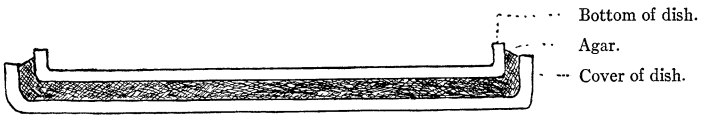

FIG. 1.

of the colony. The general characteristics are shown in Figs. 2 and 3. This type of colony develops only in the agar, not between the agar and the glass. More agar is employed than in the usual plate; otherwise a good seal between the two parts of the petri dish is not obtained. For this reason, in fishing, the needle must pierce the agar nearly vertically or the small colony is missed. One way is to bend the end of the wire so that it pierces the agar vertically, and with a circular motion break up the colony. Other bacteria give a similar colony but they are not common in the material containing fusiform bacilli. In two instances we isolated from similar colonies a motile anaerobic strepto-bacillus.

For cultivation and preservation of stock cultures we have found the most satisfactory medium to be a semisolid mixture of gelatin and agar. The constituents are as follows:

Agar	10 gms.	2 parts.
Gelatin	80 gms.	
Broth..2 per cent peptone, no salt	1,000 c.c.	
Horse serum or ascitic fluid		1 part.

Slightly alkaline to litmus is a satisfactory reaction.

Inoculation is made by stab. The advantages over agar are that the puncture closes better after inoculation, the medium does not split up on drying, and subinoculation is easy, due to the softness of the medium. Aerobic contamination is promptly noted by growth in the upper layer of the medium and a motile contaminant, likewise, would be evidenced by diffusion of the growth.

Although the various strains differ in their ability to grow on simple media, the only sure method of cultivating all strains is in the presence of serum. Horse serum is better than ascitic fluid,

possibly, because of the variations in the constitution of the latter. Freshly mixed media give much better results, probably because boiling the agar-gelatin medium drives off the contained oxygen.

As to the method of isolation, the ease with which strains may be isolated is evident from our results. Counting all the cases attempted, including those tried while perfecting our methods, we have isolated 18 strains in 42 trials. In our last seven cases we isolated a fusiform seven times.

None of the observations given have any priority. In a subsequent examination of the literature we found that Ellermann gives a picture of a similar colony and several authors speak of the

FIG. 2.—Colonies, 22 hours old.

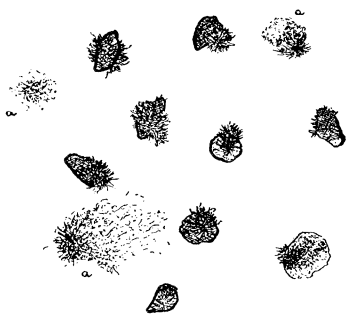

FIG. 3.—Colonies, 48 hours old. *a*, Surface or sub-surface colonies.

extensions from the colony, observations apparently made from pure cultures. No practical application of this fact was possible with the methods in use, should numerous colonies of other organisms develop. A priori we expected to find something distinctive in the colony, making this deduction from the morphology of the bacillus. The technic is given purely as a simple method requiring no apparatus and dispensing with cumbersome means of obtaining anaerobic conditions. As such it is the first simple practical method for isolation of these bacilli.

A study of the strains already isolated and the isolation of further strains for study as to their differentiation and classification, pathogenicity, relation of the fusiform bacillus to the associated spirochetes, and other biological characters is being made. The technical methods are given now in the hope that they will be of value to others interested in the subject.